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(54) Title: CD154 BLOCKADE THERAPY FOR AUTOIMMUNE DISEASES

(57) Abstract

Methods and compositions for preventing development of, delaying onset of, delaying progression of, attenuating severity of, suppressing, mitigating or treating an autoimmune disease. The disclosed methods involve use of a CD40:CD154 binding interruptor, preferably a CD154 blocking agent, such as an anti-CD154 monoclonal antibody. The methods can be used for therapy of any autoimmune disease, whether contributed to by an autoantibody response, an autoreactive T cell response, or both. The methods are useful for diseases associated with pathogen exposure, congenital diseases, hereditary diseases, and acquired diseases. The methods are particularly well-suited for therapy of idiopathic thrombocytopenia (ITP) in humans.

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CD154 BLOCKADE THERAPY FOR AUTOIMMUNE DISEASES

Related Applications

This is a continuation-in-part of: prior U.S. Provisional S.N. 60/051,072 filed June 27, 1997; and, prior U.S. Provisional S.N. 60/051,141, 60/051,483, and 60/051,484, each filed July 1, 1997. The teachings of the earlier-filed Provisional patent applications are incorporated herein by reference.

Field of the Invention

The invention relates generally to the suppression of unwanted immune responses, particularly of counter-adaptive T-lymphocyte mediated immune responses. The invention relates in particular to the prevention, treatment, suppression or reversal of counter-adaptive T-lymphocyte mediated responses to self- or autoantigens, especially autoantigens implicated in autoimmune diseases.

Background of the Invention

Autoimmunity arises when the immune system recognition of the distinction between "self" and "non-self" determinants is impaired, e.g., by exposure to a pathogen or other environmental element, by a congenital or hereditary defect, or by any other means. Autoimmune tissue destruction can be mediated predominantly by autoantibody responses, or by autoreactive T helper (T_H) cell responses, although both processes can contribute to the observed pathology of any given disease. Autoimmune lesions (sites of inflammatory or other immune response mediated tissue destruction) can occur in any body tissue, but some diseases are characterized by lesions at relatively specific body loci, whereas others are more pervasive, even systemic.

An exemplary autoimmune disease driven predominantly by autoantibody responses is idiopathic thrombocytopenia (ITP). Other representative autoantibody

mediated disease include lupus, hemolytic anemia (HA), diabetes mellitus (DM), Myasthenia gravis (MG), and rheumatoid arthritis (RA). ITP defined as a low platelet count (less than 100,000 per microliter [100 K/μL]) that cannot be explained by other causes, including drug effects or viral infections. Indeed, the majority of symptomatic and asymptomatic cases of thrombocytopenia usually turn out to be "idiopathic." Over 80% of patients with ITP have anti-platelet antibodies, with various platelet binding specificities, coating their platelets and/or circulating free in their serum. George et al. (1994), 331 N. Engl. J. Med. 1207-1250; He et al. (1994); 83 Blood 1024-1032; Berchtold et al. (1993), 81 Blood 1246-1250; Fujisawa et al. (1993), 81 Blood 2872-2877. The majority of anti-platelet antibodies are directed against platelet surface glycoproteins. About 70% are specific for GPIIb/IIIa, 20-80% are specific for GPIb/IX, and 5% for other platelet glycoproteins. He et al. (1994); 83 Blood 1024-1032; Berchtold et al. (1993), 81 Blood 1246-1250; Fujisawa et al. (1993), 81 Blood 2872-2877. These antibodies are of the IgG class and generally fix complement and bind to macrophages and other cells through the Fcγ receptor. Such antibodies enhance macrophage-mediated clearance of the platelets from circulation, leading to a reduction in the platelet count. When the platelet count drops below 30-50 K/μL, bruising, gum bleeding, hematuria, and other symptoms can develop. At platelet counts below 15-20 K/μL, the risk of intracranial hemorrhage increases substantially.

Approximately 20,000 adults are treated annually for ITP in the United States. The majority of these cases are new presentations. Most physicians agree that all patients with platelet counts <30 K/μL should be offered therapy. George et al. (1994), 331 N. Engl. J. Med. 1207-1211; George et al. (1996), 88 Blood 3-40; George et al. (1997), 126 Ann. Intern. Med. 319-326. Counts below 10-15 K/μL often constitute a medical emergency and are treated with high dose glucocorticoids and/or intravenous immunoglobulin (IVIg). The majority of patients with ITP present with platelet counts between 10 and 80 K/μL and these patients are typically treated with moderately high doses of glucocorticoids (usually prednisone, 1-1.5 mg/kg) for 2-4 weeks. George et al. (1994), 331 N. Engl. J. Med. 1207-

1211; George et al. (1997), 126 Ann. Intern. Med. 319-326. These doses of glucocorticoids are effective in raising platelet counts in nearly all cases within 1-2 weeks. As the glucocorticoid doses are lowered, platelet counts fall again.

As a result, the majority (about 85%) of patients are offered splenectomy as another treatment option (rather than remaining on moderate-dose glucocorticoids). Complete disease remissions occur in about 65% of persons who undergo splenectomy. In a small group of the remaining, non-responding patients, the presence of an accessory spleen(s) is responsible for failure of therapy; removal of the accessory spleen(s) can occasionally produce a successful outcome. Overall, approximately 20% of all patients with ITP will not achieve platelet counts >50 K/μL after splenectomy. These patients have "chronic refractory ITP." George et al. (1997), 126 Ann. Intern. Med. 319-326; McMillan (1997), 126 Ann. Intern. Med. 307-314.

For patients who have failed splenectomy, a variety of immunosuppressive medications, very high dose and/or chronic glucocorticoids, IVIg, and/or other agents are available. George et al. (1994), 331 N. Engl. J. Med. 1207-1211; George et al. (1997), 126 Ann. Intern. Med. 319-326; McMillan (1997), 126 Ann. Intern. Med. 307-314; Godeau et al. (1993), 82 Blood 1415-1421; Godeau et al. (1995), 48 Am. J. Hematol. 282-284; Anderson (1994), 330 N. Engl. J. Med. 1560-1564. However, most physicians feel that chronic, moderate-dose glucocorticoids (e.g., prednisone >15 mg/day) pose major long-term risks to their patients that outweigh the benefits of maintaining platelet counts in the "safe" (>30 K/μL) range. George et al. (1994), 331 N. Engl. J. Med. 1207-1211; George et al. (1997), 126 Ann. Intern. Med. 319-314. In some patients, relatively non-toxic agents such as colchicine, dapsone or danazol will elevate the platelet count to a small degree. George et al. (1997), 126 Ann. Intern. Med. 319-314; McMillan (1997), 126 Ann. Intern. Med. 307-314. In more refractory patients (which are the majority in the post-splenectomy population), immunosuppressive agents have shown some success. George et al. (1994), 331 N. Engl. J. Med. 1207-1211; McMillan (1997), 126 Ann. Intern. Med. 307-314.

Thus, Vinca alkaloids (vincristine, vinblastine), cyclophosphamide, or azathioprine have been used with some success. Vinca alkaloids cause bone marrow suppression and neuropathy; cyclophosphamide causes neutropenia, hemorrhagic cystitis, bladder carcinoma, bone marrow suppression, hair loss, infertility, and a variety of other side effects. Azathioprine is occasionally used, mainly as a "steroid-sparing" agent, but its efficacy is questionable and it can cause pancreatitis, hepatitis, nausea, vomiting, bone marrow suppression, and other problems. Combination chemotherapies have been used, but the risks of such therapies generally outweigh the risk of severe bleeding with platelet counts <10 K/μL. George et al. (1997), Ann. Intern. Med. 319-326. Autologous stem cell transplantation following high dose chemotherapy has been used in a limited number of patients with some success. Lim et al. (1997), 349 Lancet 475. IVIg (2-3 g/kg/dose) is often effective in acutely elevating platelet counts, but the duration of response following each course of therapy is typically <4 weeks, often only about 2 weeks, and responses often decline over time with repeated courses. George et al. (1994), 331 N. Engl. J. Med. 1207-1211; Godeau et al. (1993), 82 Blood 1415-1421. The cost of this therapy currently ranges from $8,000US to $15,000US per treatment course.

As mentioned above, another exemplary autoantibody driven disease is lupus, an autoimmune collagen vascular disease. Lupus is also described in commonly owned PCT/US97/23482, filed 31 December 1997, the teachings of which are incorporated herein by reference. In contrast to ITP, in which the autoantibody pathology is relatively specific to blood tissue, lupus is characterized by systemic inflammatory lesions of varying severity. These lesions can affect the kidneys, endocardium, lungs, joints, spleen, central nervous system (CNS), peripheral nervous system (PNS), vasculature, or any combination of the foregoing. Thus, lupus is commonly manifested as photosensitivity, fatigue, malaise, anorexia, weight loss, fever and/or inflammation of the skin, joints cardiovascular or pulmonary systems. Additional manifestations of systemic lupus erythematosus (SLE) are diverse and include polyarthritis, nephritis, proteinurea, hematological arthritis, peripheral neuropathy, neuropsychiatric abnormalities, thrombocytopenia, anemia, leukopenia, thrombocytopenia, endocarditis and pericarditis. Tan et al. (1982), 25 Arthritis Rheum.

1271-1277. Autoantibodies of various specificities have been isolated from lupus patients, including antinuclear antibodies (ANA); anti-DNA (single-stranded or double-stranded), anti-RNA, anti-ribonucleoprotein antibodies, anti-histone antibodies; anti-Ro antibodies; antibodies specific for cardiolipin and other phospholipids and/or phosphoproteins; anti-erythrocyte, anti-platelet, anti-lymphocyte and anti-neuronal antibodies. Ch. 284, Harrison's Principles of Internal Medicine, 13th ed., Isselbacher et al., eds., McGraw-Hill, Inc. (1994), pp. 1643-1648. SLE affects approximately 140,000 people in the United States and 105,000 in western Europe, predominantly women of childbearing age. About 10-20% of patients with lupus nephritis, a common manifestation of SLE, also have lupus-associated disorders of the central nervous system. These often manifest as psychological changes, but also can include vasculitis, stroke, meningitis, and meningioencephalitis. There is no cure for lupus. Currently, acute, disabling or life-threatening manifestations of lupus are treated or managed with IVIg and/or one or more of the above-discussed immunosuppressive agents, with similarly unsatisfactory outcomes.

Yet another exemplary autoimmune disease, contributed to significantly by autoreactive T cell responses, is multiple sclerosis (MS). MS is a chronic demyelinating disease, which affects some 350,000 individuals in the United States. MS is the second most frequent cause of neurologic disability in early to middle adulthood (the most frequent being trauma). The causes of MS are unclear, but appear to include both genetic and environmental contributory factors, the latter of which may include viral infection. MS produces plaques, or inflammatory lesions, in the CNS, with the periphery being largely spared. Histopathologic analysis of MS lesions reveal CNS infiltration by mononuclear cells, predominantly T lymphocytes and macrophages, as well as demyelination and gliosis (scarring). Autoreactive T cells in MS plaques are known to have specificity for myelin basic protein (MBP) or myelin proteolipid protein (PLP), and to mediate CNS inflammatory destruction. Neuronal axons with compromised myelin sheathing may display a number of functional abnormalities, such as impaired or enhanced conduction and/or crosstalk with other affected axons. Clinically, early MS symptoms often include weakness in one or more limbs, visual blurring due to optic neuritis, sensory disturbance

(e.g., parasthesia or "pins and needles" sensation, or hypesthesia or numbness), diplopia or other gaze disruption, ataxia, vertigo, incontinence. Cognitive dysfunction predominates later on, in advanced MS, and can be manifested as memory loss, depression, impaired judgement, inappropriate bouts of laughter or crying. The disease generally can be categorized either as chronic, progressive MS or relapsing/remitting MS. Current disease management strategies focus on arresting progress of the disease, using one or more of the immunosuppressant agents discussed above, and on symptomatic management using a number of agents, including antispasmodics, sedatives, antidepressants, anticholinergics, cholinergics, and dietary management. . Ch. 373, Harrison's Principles of Internal Medicine, 13th ed., Isselbacher et al., eds., McGraw-Hill, Inc. (1994), pp. 2287-2295. Other approaches to MS management involve the use of beta-interferons (IFN βs), such as AVONEX. However, these antiviral therapeutic agents can generate flu-like side effects, which operate as a disincentive to continued therapy.

There is accordingly a need for improved or more effective immunosuppressive or immunomodulatory treatments for autoimmunity, whether contributed to by autoantibody responses or by autoreactive T lymphocyte responses. In particular, there is a need for treatments that do not require pan-T cell immunosuppression, i.e., treatments that do not leave the recipient vulnerable to malignancies, opportunistic infection or the toxicities associated with chronic glucocorticoid usage. There is a poignant need for improved clinical strategies to manage (attenuate or ameliorate) diseases such as: lupus including SLE, ITP, HA, DM, MG, and RA. Similarly, There is a poignant need for improved strategies to manage diseases such as: MS, anti-phospholipid syndrome (APS), Crohn's disease, and inflammatory bowel disease (IBD).

Summary of the Invention

It is an object of this invention to provide an immunomodulatory agent that mitigates counter-adaptive T cell responses without the need for pan-T cell immunosuppression or chronic glucocorticoid use. Another object is to provide an immunomodulatory agent that inhibits or suppresses autoantibody production. Thus, a

further object is to provide an immunomodulatory agent that interrupts delivery of a costimulatory signal to activated T cells. Another object is to provide an immunomodulatory agent that inhibits, prevents or delays onset of an autoimmune disease, particularly an autoantibody driven disease. Still another object is to provide an immunomodulatory agent that delays progression of an autoimmune disease, mitigates the disease, treats it or suppresses its effects or manifestations. A particular object is to provide a CD40:CD154 binding interruptor, such as a CD154 blocking agent, for use in therapy, particularly for use in therapy to mitigate, delay or reverse autoimmune diseases, including ITP, all forms of lupus including SLE, HA, DM, MG, RA, MS, APS, Crohn's disease, IBD, and the like.

The present invention rests on the discovery that use of a CD40:CD154 binding interruptor, such as a CD154 blocking agent, prevents, delays, attenuates, mitigates, suppresses, treats or reverses counter-adaptive immune system responses to self-components, such as autoantigens, *without* the need for pan-suppression of a subject's immune system. More precisely, the present invention rests on the discovery that a CD154 blocking agent beneficially blocks or interferes with counter-adaptive, self-destructive effects of numerous human autoimmune diseases, regardless of whether the disease is associated with exposure to a pathogen, is congenital or acquired, or is associated with a hereditary risk factor.

The invention accordingly provides methods and compositions for immunomodulatory therapy for blockade or inhibition of an inappropriate, self-directed immune response, irrespective of whether the response is an autoantibody response or an autoreactive T cell response. A first method prevents development of an autoimmune disease. A second method delays onset of the disease. A third method delays progression of the disease. A fourth method attenuates (detectably downmodulates) severity of the disease. A fifth method suppresses one or more effects or manifestations of the disease. A sixth method mitigates or abrogates the disease. A seventh method treats (improves clinical status or stage of) the disease. All of the foregoing methods involve treating a

subject at risk of, or afflicted with, the disease with a CD40:CD154 binding interruptor, by which is meant any agent that interrupts the binding of CD40 Ligand (CD40L, also known as CD154 or the 5c8 antigen, and sometimes referred to in the art as gp39 or TRAP) to its counter or cognate receptor (CD40). Preferably, the binding interruptor is a CD154 (CD40L) blocking agent, by which is meant any agent that binds to CD154 and prevents or interferes with its binding to its counter receptor(s). An exemplary CD154 blocking agent is a monoclonal antibody (MAb), particularly one having the antigen-specific binding characteristics of the 5c8 MAb disclosed in U.S. Patent 5,474,771, the teachings of which are incorporated herein by reference. The present methods are suitable for human or animal (e.g., primate) therapy.

The present invention accordingly provides means for treating autoimmune disease manifestations (e.g., lesions) affecting any body tissue, solid organ or organ system. For example, the present methods can be used for treatment of lesions affecting cutaneous, cardiac, pericardial, endocardial, vascular lining or wall, blood (e.g., erythrocytes, platelets), blood-forming (e.g., marrow, spleen), endocrine (e.g., pancreatic, thyroid), gastrointestinal tract (e.g., bowel), respiratory tract (e.g., nasopharyngeal, sinus, bronchial, lung), renal, central nervous system (CNS), peripheral nervous system (PNS), muscular or skeletal joint (e.g., articular cartilage; synovial lining or fluid) tissue. Accordingly, the present methods can be used for treatment of any autoimmune disease, including atopic dermatitis, any form of lupus (including cutaneous lupus (discoid lupus erythematosis), and any extracutaneous type of lupus, including systemic lupus erythrematosus (SLE) acute lupus, lupus annularis, lupus discretus, lupus lymphaticus, lupus papollomatis, lupus psoriasis, lupus vulgaris, lupus sclerosis, neonatal lupus erythematosus and drug-induced lupus), anti-phospholipid syndrome (APS), hemolytic anemia (HA), idiopathic thromocytopenia (ITP), thyroiditis, diabetes mellitus (DM), thyroiditis, inflammatory bowel disease (IBD), Crohn's disease, rhinitis, demyelinating diseases such as multiple sclerosis (MS), myasthenia gravis (MG), and rheumatoid arthritis (RA).

The foregoing and other objects, features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments.

Detailed Description of the Invention

T cell activation, and immunological processes dependent thereon, requires both T cell receptor (TCR) mediated signals and concurrently delivered costimulatory signals. An important costimulatory signal is delivered by the ligation of CD40 on an antigen-presenting cell (APC), such as a dendritic cell, B cell, or macrophage, by CD40L (CD154) on a T cell. Grewal et al. (1996), 273 Science 1864-1867. Human CD40 is a 50 kD cell surface protein expressed on APCs, including mature B cells, as well as on macrophages, activated endothelial cells and activated smooth muscle cells. CD40 belongs to a class of receptors involved in programmed cell death, including Fas/CD95 and the tumor necrosis factor alpha (TNFα) receptor. Human CD154 (CD40L) is a 32 kD type II membrane glycoprotein with homology to TNFα that is expressed, transiently, primarily on activated T cells (CD154 also can be expressed on other activating cell types). CD40:CD154 binding has been shown to be required for mounting T helper cell (T_H) immune responses, including all T cell-dependent antibody responses. Lederman et al. (1992), 175 J. Exp. Med. 1091-1101; Alderson et al. (1993), 178 J. Exp. Med. 669-674; Van den Eertwegh et al. (1993), 178 J. Exp. Med. 1555-1565; Shu et al. (1995), 25 Eur. J. Immunol. 1125-1128; Foy et al. (1996), 14 Ann. Rev. Immunol. 591-617; Stout et al. (1996) 156 J. Immunol. 8-11; Van Kooten et al. (1996), 61 Adv. Immunol. 1-77. In particular, CD40:CD154 binding provides anti-apoptotic and/or lymphokine stimulatory signals.

The importance of CD40:CD154 binding in promoting T cell dependent biological responses was more fully appreciated when it was discovered that X-linked hyper-IgM syndrome (X-HIGM) in humans is the phenotype resulting from genetic lack of functional CD154. Affected individuals have normal or high IgM levels, but fail to produce IgG, IgA or IgE antibodies, and suffer from recurrent, sometimes severe, bacterial and parasitic infections, as well as an increased incidence of lymphomas and abdominal cancers.

Notarangelo et al. (1992), 3 Immunodeficiency Rev. 101-122; Aruffo et al. (1993), 72 Cell 291-300; Callard et al. (1993), 14 Immunol. Today 559-564; Ramesh et al. (1994), 138 Immunol. Rev. 87-104. A similar phenotype is observed in non-human animals rendered nullizygous for the gene encoding CD154 (knockout animals). B cells of CD154 nullizygotes can produce IgM in the absence of CD40L:CD154 binding, but are unable to undergo isotype switching, or to survive normally after affinity maturation. Histologically, lymph node germinal centers fail to develop properly, and memory B cells are absent or poorly developed. Functionally, these defects contribute to a severe reduction or absence of a secondary (mature) antibody response. Defects in cellular immunity are also observed, manifested by an increased incidence of bacterial and parasitic infections. Many of these cell-mediated defects are reversible by administration of IL-12 or IFN-gamma. Renshaw et al. (1994), 180 J. Exp. Med. 1889-1900; Xu et al. (1994), 1 Immunity 423-431. These observations substantiate the view that normal CD40:CD154 binding promotes the development of Type I T-helper cell immunological responses.

Blockade of the CD40:CD154 interaction during immunization with protein antigens can specifically block the antibody response to that antigen in mice. Foy et al. (1993), 178 J. Exp. Med. 1567-1575; Van den Ertweigh et al. (1993), 178 J. Exp. Med. 1555-1565. For example, anti-CD154 antibodies can block the induction of anti-collagen antibodies in collagen-induced arthritis. Durie et al. (1993), 261 Science 1328-1330. Anti-CD154 antibodies can reduce anti-dsDNA and anti-nucleosomal autoantibodies in mice with spontaneous lupus. Mohan et al. (1995), 154 J. Immunol. 1470-1480; Gerritse et al. (1996), 93 Proc. Natl. Acad. Sci. USA 2499-2504. In addition, anti-CD154 antibodies can reduce symptoms in mice with experimental autoimmune encephalomyelitis (EAE), a model of MS. Similar results have been reported in rodent models of graft-versus-host-disease (Durie et al. (1994), 94 J. Clin. Invest. 1333-1338), mercuric chloride induced glomerulonephritis (Biancone et al. (1995), 48 Kidney Intl. 458-468), and inflammatory bowel disease.

CD40:CD154 blockade thus may provide potentially powerful therapies for attenuating or ameliorating unwanted, self-reactive immune responses, particularly in the context of autoimmune diseases. However, despite numerous reports of promising results, studies performed in rodent models of induced counter-adaptive immunological disease (e.g., induced autoimmunity) have correlated poorly with the outcome of testing in actual disease contexts, or even in larger animal preclinical model systems (e.g., primates).

Disclosed herein are protocols for assessing the effects of a preferred CD154 blocking agent, a humanized MAb having the antigen-specific binding properties of MAb 5c8 (Lederman et al. (1992), 175 J. Exp. Med. 1091-1101), in human clinical trials for idiopathic thrombocytopenia (ITP), an exemplary autoimmune disease for which no well-established preclinical model system is available. The disclosed protocols are expected to yield results strongly suggesting that CD154 blockade therapy is not limited to effectiveness for ITP, but will also effectively suppress or attenuate manifestations of many other autoimmune diseases, particularly autoantibody-driven diseases.

The following discussion illustrates and exemplifies the variety of contexts and circumstances in which the invention can be practiced, as well as providing proof-of-principle studies involving specific embodiments of the invention.

Subjects for Treatment

The invention can be used for treatment or prophylaxis of any mammalian subject in need of, or already receiving, immunosuppressive therapy for unwanted immune system responses to self-components, such as autoantigens or autoreactive T cells. Preferably, the subject mammal is a primate, more preferably a higher primate, most preferably a human. In other embodiments, the subject may be another mammal in need of immunomodulatory therapy, particularly a mammal of commercial importance, or a companion animal or other animal of value, such as a member of an endangered species. Thus, subjects also include, but are not limited to, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice.

Subjects are afflicted with, or at risk of, an autoimmune disease. Subjects may have autoimmune pathology affecting any body tissue or organ, including skin, heart, pericardium, endocardium, vasculature, any blood component (e.g., erythrocytes, platelets), blood-forming tissue (e.g., marrow, spleen), endocrine tissue or organs (e.g., pancreas, thyroid), gastrointestinal tract (e.g., bowel), respiratory tract (e.g., lung), kidney, central nervous system (CNS), peripheral nervous system (PNS), muscle and skeletal joints. Thus, the invention can be practiced on any patient who presents with symptoms, manifestations or significant risk factors for atopic dermatitis, any form of lupus (including cutaneous lupus (discoid lupus erythematosis), and any extracutaneous type of lupus, including systemic lupus erythrematosus (SLE) acute lupus, lupus annularis, lupus discretus, lupus lymphaticus, lupus papollomatis, lupus psoriasis, lupus vulgaris, lupus sclerosis, neonatal lupus erythematosus and drug-induced lupus), anti-phospholipid syndrome (APS), hemolytic anemia (HA), idiopathic thromocytopenia (ITP), thyroiditis, diabetes mellitus (DM), thyroiditis, inflammatory bowel disease (IBD), Crohn's disease, rhinitis, myasthenia gravis (MG), rheumatoid arthritis (RA) and demyelinating diseases such as multiple sclerosis (MS),. For example, subjects presenting with manifestations of ITP generally have a platelet count of less than 150,000 per cubic milliliter of blood, with the diminished platelet count having persisted for a minimum of three months without other clinical findings that could account for it.

Exemplary CD40:CD154 Binding Interruptors

Therapeutic compounds useful for practice of the invention include any compound that blocks the interaction of cell surface CD40 (e.g., on B cells) with CD40L (CD154) expressed, e.g., on the surface of activated T cells. CD40:CD154 binding interruptor compounds, such as CD154 blocking agents, that are specifically contemplated include polyclonal antibodies and monoclonal antibodies (MAbs), as well as antibody derivatives such as chimeric molecules, humanized molecules, molecules with reduced effector functions, bispecific molecules, and conjugates of antibodies. In a preferred embodiment, the antibody has substantially the same antigen-specific binding characteristics as MAb

5c8, as described in U.S. Patent 5,474,771, the disclosure of which is hereby incorporated by reference. In a currently highly preferred embodiment, the antibody is a humanized 5c8 (hu5c8). Other known antibodies against CD154 include antibodies ImxM90, ImxM91 and ImxM92 (disclosed by Immunex Corp., Seattle WA), an anti-CD40L MAb commercially available from Ancell (clone 24-31, catalog # 353-020, Bayport, MN), and an anti-CD154 MAb commercially available from Genzyme (Cambridge, MA, catalog # 80-3703-01). Also commercially available is an anti-CD154 MAb from PharMingen (San Diego, catalog #33580D). Numerous additional anti-CD154 antibodies have been produced and characterized (see, e.g., WO 96/23071 of Bristol-Myers Squibb, the specification of which is hereby incorporated by reference).

The invention also includes use of CD154 blocking agents that are derived from, or engineered from the above-mentioned and equivalent MAbs, such as complete Fab fragments, $F(ab')_2$ compounds, V_H regions, F_V regions, single chain antibodies (see, e.g., WO 96/23071), polypeptides, fusion constructs of polypeptides, fusions of CD40 (such as CD40Ig, as in Hollenbaugh et al. (1995), 188 J. Immunol. Meth.1-7, which is hereby incorporated by reference), and small molecule compounds such as small semi-peptidic compounds or non-peptide compounds, all capable of blocking or interrupting CD40:CD154 binding. Procedures for designing, screening and optimizing small molecules are provided in PCT/US96/10664, filed June 21, 1996, the specification of which is hereby incorporated by reference.

Thus, the invention can be practiced with MAb-derived, CD154 blocking agents created using standard recombinant DNA techniques (Winter and Milstein (1991), 349 Nature 293-99). One class of such CD154 blocking agents includes chimeric antibodies, or fusion proteins constructed by joining nucleic acid encoding the antigen binding domain of a non-human mammalian antibody (e.g., a mouse or rat antibody) of desired specificity to nucleic acid encoding a human immunoglobulin (Ig) constant region. Cabilly et al., United States Pat. No. 4,816,567; Morrison et al. (1984), 81 Proc. Natl. Acad. Sci. USA 6851-55. Chimeric antibody polypeptides expressed from such constructs generally have lower

immunogenicity, when used for human therapy or prophylaxis, than the non-human antibody from which the chimera was derived. A second class of such CD154 blocking agents includes recombinant "humanized" or "primatized"antibodies. Humanized or primatized antibodies are antibodies are genetically engineered from non-human mammalian antibodies having the desired specificity, by replacing some or all of the codons for amino acids not required for antigen binding with codons for amino acids from corresponding regions of a human or primate Ig light or heavy chain gene. That is, they are chimeras comprising mostly human immunoglobulin sequences into which the regions responsible for antigen specific binding have been genetically inserted (see, e.g., PCT patent application WO 94/04679). Humanized antibodies generally have even lower immunogenicity in vivo than chimeric antibodies. Currently, a humanized MAb having substantially the same antigen specificity as MAb 5c8 (herein, hu5c8) is preferred for practice of the invention.

Another class of MAb-derived CD154 blocking agents useful in the invention includes human antibodies, which can be produced in transgenic nonhuman mammals, into whom one or more human immunoglobulin transgenes have been integrated. Such animals may be used as a source for splenocytes for producing human hybridomas, as described in U.S. 5,569,825.

Of course, any antigen-specific binding fragment of one of the foregoing MAbs or MAb derived therapeutic agent can be used in the present invention, provided that the fragment is sufficiently large to sterically impede CD154 binding to its counter-receptor. Thus, MAb fragments and univalent MAbs can be used. Univalent antibodies comprise a heavy chain/light chain dimer bound to the Fc (or stem) region of a second heavy chain. "Fab region" refers to those portions of the chains which are roughly equivalent, or analogous, to the sequences which comprise the Y branch portions of the heavy chain and to the light chain in its entirety, and which collectively (in aggregates) have been shown to exhibit antibody activity. A Fab protein includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers which correspond to the two branch

segments of the antibody Y, (commonly known as $F(ab)_2$), whether any of the above are covalently or non-covalently aggregated, so long as the aggregation is capable of selectively reacting with a particular antigen or antigen family.

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody may be increased by mutagenesis based on molecular modeling (Queen et al., Proc. Natl. Acad. Sci. 86:10029-33, 1989; PCT patent application WO 94/04679). It may be desirable to increase or to decrease the affinity of the antibodies for CD154, depending on the targeted tissue type or the particular treatment schedule envisioned. This may be done utilizing phage display technology (see, e.g., Winter et al., Ann. Rev. Immunol. 12:433-455, 1994; and Schier et al., J. Mol. Biol. 255:28-43, 1996, which are hereby incorporated by reference). For example, it may be advantageous to treat a patient with constant levels of antibodies with reduced affinity for CD154 for semi-prophylactic treatments. Likewise, antibodies with increased affinity for CD154 may be advantageous for short-term treatments.

Routes of Administration

The CD40:CD154 binding interruptors, including CD154 blocking agents, used in the invention can be administered in any manner which is medically acceptable. Depending on the specific circumstances, local or systemic administration may be desirable. Preferably, the agent is administered via a parenteral route such as by an intravenous, intraarterial, subcutaneous, intramuscular, intraorbital, intraventricular, intraperitoneal, subcapsular, intracranial, intraspinal, or intranasal injection, infusion or inhalation. At present, intravenous, intramuscular and subcutaneous routes are preferred. The agent also can be administered by implantation of an infusion pump, or a biocompatible or bioerodable sustained release implant, into the recipient host, either before or after implantation of donor tissue. Alternatively, certain compounds of the

invention, or formulations thereof, may be appropriate for oral or enteral administration. Still other compounds of the invention will be suitable for topical administration.

In further embodiments, the CD40:CD154 binding interruptor is provided indirectly to the recipient, by administration of a vector or other expressible genetic material encoding the interruptor. The genetic material is internalized and expressed in cells or tissue of the recipient, thereby producing the interruptor in situ. For example, a suitable nucleic acid construct would comprise sequence encoding one or more of the MAb 5c8 immunoglobulin (Ig) chains as disclosed in U.S. Pat. 5,474,771. Other suitable constructs would comprise sequences encoding chimeric or humanized versions of the MAb 5c8 Ig chains or antigen-binding fragments thereof. Still other suitable constructs would comprise sequences encoding part or all of other CD154-specific MAbs. The construct is delivered systemically or locally, e.g., by local injection, infusion or implantation to a desired site.

Alternatively, the vector or other genetic material encoding the interruptor is internalized within a suitable population of isolated cells to generate interuptor-producing host cells. These host cells then are implanted or infused into the subject, either locally or systemically, to provide in situ production of the CD40:CD154 binding interruptor. Appropriate host cells include cultured cells, such as immortalized cells, as well as cells obtained from the recipient (e.g., peripheral blood or lymph node cells, such as natural killer (NK) cells).

Formulation

In general, the compound(s) used in practice of the invention are suspended, dissolved or dispersed in a pharmaceutically acceptable carrier or excipient. The resulting therapeutic composition does not adversely affect the recipient's homeostasis, particularly electrolyte balance. Thus, an exemplary carrier comprises normal physiologic saline (0.15M NaCl, pH 7.0 to 7.4). Another exemplary carrier comprises 50 mM sodium phosphate, 100 mM sodium chloride. Many other acceptable carriers are well known in the art and are described, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., 1990. Acceptable carriers can include biocompatible, inert or

bioabsorbable salts, buffering agents, oligo- or polysaccharides, polymers, viscosity-improving agents, preservatives, and the like.

Any CD40:CD154 binding interruptor, such as a CD154 blocking agent, that is used in practice of the invention is formulated to deliver a pharmaceutically-effective or therapeutically-effective amount or dose, which is an amount sufficient to produce a detectable, preferably medically beneficial effect on the subject. Medically beneficial effects would include preventing, delaying or attenuating deterioration of, or detectably improving, the subject's medical condition. As an example, a medically beneficial effect on a subject afflicted with ITP would include an increase in platelet count, preferably to a level between about 50,000 and 150,000 per cubic milliliter. Thus, an effective amount of a therapeutic compound of the invention, such as a CD154 blocking agent, is any amount which produces a detectable, preferably sustained, increase in platelet count. An optimal effective amount is one which returns the platelet count to normal levels.

Similarly, for a subject afflicted with atopic dermatitis, an effective amount is any which detectably eases or prevents worsening of erythema, edema or other skin irritation. For a subject afflicted with lupus, an effective amount is any which detectably eases or prevents worsening of nephritis, pericarditis, endocarditis, vasculitis, sclerosis, or any other lupus-associated pathology, including neurological pathologies and abdominal inflammation, particularly splenic inflammation or fibrosis. For an APS subject, an effective amount is any which detectably eases or prevents worsening of valvular disease, pericarditis, endocarditis or vasculitis. For an HA subject, an effective amount is any which detectably eases or prevents worsening of anemia. For an ITP subject, an effective amount is any which detectably eases or prevents worsening of bleeding events. For a DM subject, an effective amount is any which detectably eases or prevents worsening of dependence on insulin to maintain glucose homeostasis. For an IBD or Crohn's disease subject, an effective amount is any which detectably eases or prevents worsening of bowel stenosis, anorexia, malabsorbtion or other associated pathology. For a demyelinating disease subject, such as an MS patient, an effective amount is any which detectably eases

or prevents worsening of any associated CNS pathology. For an MG subject, an effective amount is any which detectably eases or prevents worsening of neuromuscular pathology. Finally, for an RA subject, an effective amount is any which detectably eases or prevents worsening of joint swelling, pain or degradation.

Dosages and Frequency of Treatment

The amount of and frequency of dosing for any particular compound to be used in practice of the invention is within the skills and clinical judgement of ordinary practitioners of the medical arts, such as physicians. The general dosage and administration regime is established by preclinical and clinical trials, which involve extensive but routine studies to determine effective, e.g., optimal, administration parameters for the desired compound. Even after such recommendations are made, the practitioner will often vary these dosages for different subjects based on a variety of considerations, such as the subject's age, medical status, weight, sex, and concurrent treatment with other pharmaceuticals. Determining effective dosage and administration regime for each CD40:CD154 binding interruptor used in the invention is a routine matter for those of skill in the pharmaceutical and medical arts. The dosage amount and timecourse of should be sufficient to produce a clinically beneficial change in one or more indicia of the subject's health status. For some subjects, a single dose may be sufficient to produce the desired response, while in others, daily, biweekly, weekly, bimonthly or monthly doses may be required. Exemplary timecourse and dosage regimes are set forth in the Examples included herein.

To further exemplify dosing considerations for an anti-CD154 compound, the following examples of administration strategies are given for an anti-CD154 MAb. The dosing amounts could easily be adjusted for other types of CD154 blocker compounds. In general, single dosages of between about 0.05 and about 50 mg/kg subject body weight are contemplated, with dosages most frequently in the 1-20 mg/kg range. To initiate CD154 blockade therapy prophylactically, when the subject is in remission, or for emergency therapy of acute disease, an effective dose of MAb ranges from about 1 mg/kg body weight to about 20 mg/kg body weight, administered daily or at intervals ranging from two to five

days, for a period of up to about three weeks. Therapy can be maintained by administering the MAb intermittently thereafter, in dosages ranging from about 0.1 mg/kg body weight to about 20 mg/kg body weight, e.g., from about 0.2 mg/kg to about 10 mg/kg. For maintenance purposes, the interdose interval may range from about one week up to about three months. At present, a one-month (four week) interdose interval is preferred.

Generally, therapy is commenced with low doses of anti-CD154 MAb. For example, an initial dose of MAb contains between about 1.0 mg and 30 mg MAb for a 70 kg subject. For repeated administrations (e.g., during the load phase of a load/maintenance regime), doses can be administered on successive days, every two to six days, once weekly, once every two or three weeks, or once monthly, until the desired effect on the subject's health status is achieved. Therapy can be suspended until disease manifestations are again observed, or if deemed prudent, can be maintained, e.g., by administering MAb every three weeks, on a monthly basis, a bimonthly basis, quarterly, semiannually or annually. Reappearance of disease manifestations can be treated by repeating the initial dosage series, or by a bolus administration of MAb (e.g., 100 mg).

CD154 blockade therapy can be practiced, if desired, serially or in combination with conventional immunosuppression therapy. A conventional immunosuppressant agent (e.g., a corticosteroid or calcineurin inhibitor) can be co-administered at any point during CD154 blockade therapy deemed prudent by the practitioner. Alternatively, a CD154 blocking MAb may be conjugated to a conventional agent. This advantageously permits the administration of the conventional agent in an amount less than the conventional dosage, for example, less than about 50% of the conventional dosage, when the agent is administered as monotherapy. Accordingly, the occurrence of many side effects associated with that agent should be avoided. Thus, according to this invention, CD154 blocking MAbs can be used together with other agents targeted at B cells, such as anti-CD19, anti-CD28 or anti-CD20 antibody (unconjugated or radiolabeled), IL-14 antagonists, LJP394 (LaJolla Pharmaceuticals receptor blocker), IR-1116 (Takeda small molecule) and anti-Ig idiotype monoclonal antibodies. Alternatively, the combinations may include T cell/B cell

targeted agents, such as CTLA4Ig, IL-2 antagonists, IL-4 antagonists, IL-6 antagonists, receptor antagonists, anti-CD80/CD86 monoclonal antibodies, TNF, LFA1/ICAM antagonists, VLA4/VCAM antagonists, brequinar and IL-2 toxin conjugates (e.g., DAB), prednisone, anti-CD3 MAb (OKT3), mycophenolate mofetil (MMF), cyclophosphamide, and other immunosuppressants such as calcineurin signal blockers, including without limitation, tacrolimus (FK506). Combinations may also include T cell targeted agents, such as CD4 antagonists, CD2 antagonists and IL-12.

Clinical Model Systems for Evaluating CD40:CD154 Interruptor Treatment Regimes

Currently preferred, exemplary model systems for testing efficacy of a CD40:CD154 interrupting compound (e.g., an anti-CD40L compound or a CD154 blocking agent, such as a MAb having the specificity of MAb 5c8) are set forth below. In each system, routine modifications or adaptations can be made, to tailor the techniques as needed to assess the effects of any desired CD40:CD154 interrupting compound on the status of autoimmune disease. Some exemplary modifications are mentioned below; however, many other appropriate modifications will be apparent to the skilled practitioner and are contemplated herein. In particular, the protocol can be routinely adapted for testing efficacy of a CD154 blocking agent in any other autoimmune disease, e.g., by substituting appropriate subject inclusion criteria, evaluations, analyses, and therapeutic endpoints. For example, to adapt the study design for testing efficacy for SLE subjects, an appropriate therapeutic endpoint could be a measurable effect on hematuria, proteinuria, glomerular filtration rate (GFR), serum creatinine, serum complement level, or serum titer of an autoantibody (e.g., an anti-DNA antibody). Similarly, to adapt the study design for testing efficacy for DM subjects, an appropriate therapeutic endpoint could be a measurable effect on frequency of insulin use, circulating glucose level, performance in a glucose tolerance test, or serum titer of an autoantibody reactive with pancreatic islet beta (β) cells.

Randomized, double-blind, Idiopathic Thrombocytopenia (ITP) study.

This study involves three cohorts of at least 12 subjects (anticipating some dropouts during the course of the study), such that at least 36 subjects will complete the study. Each

randomized 12 subject cohort will receive a humanized 5c8 MAb (hu5c8) at a dose of 0 mg/kg, 3 mg/kg or 10 mg/kg. Subjects will receive 6 doses given every four weeks, not to exceed a 24 week study period. The primary endpoint established for this study is to determine the number of subjects achieving and maintaining platelet counts >30 K/μL. There are two secondary endpoints: to determine the number of subjects achieving a >50% reduction in anti-platelet antibody titer, including *any* reduction in need for IVIg (emergent treatment), and (2) to determine *any significant* reduction in cumulative steroid dosage, including the reduction in study dropouts for splenectomy, as well as *any* reduction in clinically overt bleeding (major and minor episodes).

Inclusion Criteria. Candidates will be eligible for entry into this study if all of the following inclusion criteria are met within 14 days prior to study drug administration, unless otherwise specified:

1) Must be males or females between the ages of 18 to 75 years, inclusive.

2) Female subjects must be post-menopausal or surgically sterile, or using contraception. In particular, all female subjects must have a negative pregnancy test on predosing evaluation.

3) Must carry a diagnosis of ITP within the 6 months prior to initiation of dosing in this study *and* must have had a platelet count <50,000/μL on at least one occasion between 1 and 6 months, inclusive, prior to study drug administration.

4) Must have pre-dosing laboratory findings as follows:

Creatinine	<2.0 mg/dL
BUN	<40 mg/dL
Sodium	≥130 mEq/L but ≤150 mEq/L
Potassium	≥3.0 mEq/L but ≤5.5 mEq/L
WBC	>2.5 $X10^3/mm^3$ but <12.0 $X10^3/mm^3$

7) Must have a physical examination and an electrocardiogram (ECG) essentially free of any clinically significant abnormality.

8) Must have pre-dosing laboratory findings without clinically significant abnormal values for: hematocrit, white blood count (WBC) and differential, serum blood urea nitrogen (BUN), creatinine, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), prothrombin time (PT), activated partial thromboplastin time (aPTT).

9) Unless contraindicated, must have received vaccinations for: influenza (within the previous 2 years), pneumococcus (within the previous 10 years), and tetanus toxoid (within the previous 10 years).

Evaluation. The primary analysis, for subjects who complete the full 24 weeks of dosing, will be of the proportion of subjects maintaining platelet counts >30 K/μL. This proportion will be analyzed by logistic regression, and a component due to dose response will be isolated from the" between treatment" variation. Each hu5c8 dose level will be compared with control. The secondary analyses, for subjects who complete the full 24 weeks of dosing, will be of the mean platelet count and/or the mean change (or percentage change) for each subject. Baseline will be defined as the mean of results from patient samples taken within 14 days and 1 hour prior to the first dose of hu5c8. In addition, the proportion of subjects who require any IVIg as emergent treatment will be analyzed.

The above results will be analyzed by one-way analysis of variance or logistic regression with a component due to dose response isolated from the "between treatment" variation. Each dose will be compared with control. Additionally, the time to failure of therapy, as defined by the first occurrence of a platelet count <30 K/μL, will be summarized by Kaplan-Meier curves, and analyzed by proportional hazard models to examine dose response, and to compare each dose with control. This will also be done for the first occurrence of a platelet count <20 K/μL.

Expected Results. hu5c8 treatment, at one or both dosage levels, is expected to produce a stable restoration of platelet count, e.g., a persistent count of <30 K/μL, in a statistically significant proportion of subjects.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative of, rather than limiting on, the invention disclosed herein. Scope of the invention thus is indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

What is claimed is:

1. A method of preventing development of an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

2. A method of delaying onset of an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

3. A method of delaying progression of an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

4. A method of attenuating severity of an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

5. A method of suppressing effects of an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

6. A method of mitigating an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

7. A method of treating an autoimmune disease in a subject, comprising the step of administering an effective amount of a CD40:CD154 binding interruptor to the subject.

8. A method according to claim 1, 2, 3, 4, 5, 6, or 7 wherein the CD40:CD154 binding interruptor is a CD154 (CD40L) blocking agent.

9. A method according to claim 8, wherein CD154 blocking agent is a monoclonal antibody.

10. A method according to claim 9, wherein the monoclonal antibody has the antigen-specific binding characteristics of the 5c8 antibody produced by ATCC Accession No. HB 10916.

11. A method according to claim 1, 2, 3, 4, 5, 6, or 7, wherein the subject is a primate.

12. A method according to claim 11, wherein the subject is a human.

13. A method according to claim 1, 2, 3, 4, 5, 6, or 7, wherein the autoimmune disease is associated with exposure to a pathogen.

14. A method according to claim 1, 2, 3, 4, 5, 6, or 7, wherein the autoimmune disease is associated with a congenital or hereditary risk factor.

15. A method according to claim 1, 2, 3, 4, 5, 6, or 7, wherein the autoimmune disease is contributed to by an autoantibody response.

16. A method according to claim 1, 2, 3, 4, 5, 6, or 7, wherein the autoimmune disease is contributed to by an autoreactive T cell response.

17. A method according to claim wherein the autoimmune disease affects cutaneous, cardiac, pericardial, endocardial, vascular, blood, blood-forming, endocrine, gastrointestinal tract, respiratory tract, renal, central nervous system (CNS), peripheral nervous system (PNS), muscular, or skeletal joint tissue.

18. A method according to claim 17 wherein the autoimmune disease is atopic dermatitis.

19. A method according to claim 17 wherein the autoimmune disease is anti-phospholipid syndrome,

20. A method according to claim 17 wherein the autoimmune disease is hemolytic anemia.

21. A method according to claim 17 wherein the autoimmune disease is idiopathic thrombocytopenia (ITP).

22. A method according to claim 17 wherein the autoimmune disease is thyroiditis.

23. A method according to claim 17 wherein the autoimmune disease is diabetes mellitus.

24. A method according to claim 17 wherein the autoimmune disease is inflammatory bowel disease.

25. A method according to claim 17 wherein the autoimmune disease is Crohn's disease.

26. A method according to claim 17 wherein the autoimmune disease is rhinitis.

27. A method according to claim 17 wherein the autoimmune disease is lupus.

28. A method according to claim 17 wherein the autoimmune disease is a demyelinating disease.

29. A method according to claim 17 wherein the autoimmune disease is multiple sclerosis.

30. A method according to claim 17 wherein the autoimmune disease is myasthenia gravis.

31. A method according to claim 17 wherein the autoimmune disease is rheumatoid arthritis.

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 98/13284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 09812 A (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 27 May 1993 see claims 68-76 ---	1-31
X	WO 97 20063 A (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, ET AL.) 5 June 1997 see "first series of experiments" see claims 82,87,88,91,93,95,97 ---	1-17,28, 29,31
X	WO 97 17446 A (IDEC PHARMACEUTICAL CORPORATION) 15 May 1997 see claims --- -/--	1-9, 11-31

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 1998	21/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 98/13284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 40246 A (TRUSTEES OF DARTMOUTH COLLEGE ET AL.) 19 December 1996 see examples see claims	1-9,11, 12,16, 17,28,29
X	WO 96 23071 A (BRISTOL-MYERS SQUIBB COMPANY) 1 August 1996 see claims	1-9, 11-17, 23, 27-29,31
X	K. GERRITSE ET AL.: "CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE U.S.A., vol. 93, no. 6, 19 March 1996, pages 2499-2504, XP002080038 Washington, DC, USA cited in the application see abstract see page 2504, left-hand column, line 3 - line 18	1-9, 11-17, 28,29
X	J. BUHLMANN ET AL.: "Therapeutic potential for blockade of the CD40 ligand, gp39." JOURNAL OF CLINICAL IMMUNOLOGY, vol. 16, no. 2, March 1996, pages 83-89, XP002080039 New York, NY, USA see page 85, right-hand column, line 3 - page 87, right-hand column, line 3	1-9, 11-17, 27-29,31
X	A. DESAI-MEHTA ET AL.: "Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production." THE JOURNAL OF CLINICAL INVESTIGATION, vol. 97, no. 9, 1 May 1996, pages 2063-2073, XP002080040 New York, NY, USA see the whole document	1-9, 11-17,27
X	E. RESETKOVA ET AL.: "Antibody to gp39, the ligand for CD40 significantly inhibits the humoral response from Graves' thyroid tissues xenografted into severe combined immunodeficient (SCID) mice." THYROID, vol. 6, no. 4, August 1996, pages 267-273, XP002080041 New York, NY, USA see abstract	1-9, 11-17

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INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 98/13284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. EARLY ET AL.: "Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand White mice." THE JOURNAL OF IMMUNOLOGY, vol. 157, no. 7, 1 October 1996, pages 3159-3164, XP002080042 Baltimore, MD, USA see abstract see page 3164, left-hand column, line 14 - line 26 -----	1-9, 11-17,27

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 13284

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-31
are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/US 98/13284

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9309812 A	27-05-1993	US 5474771 A	12-12-1995
		AU 675922 B	27-02-1997
		AU 3141993 A	15-06-1993
		CA 2123224 A	27-05-1993
		EP 0614374 A	14-09-1994
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WO 9720063 A	05-06-1997	AU 1143897 A	19-06-1997
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